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An Endogenous SCP-Related Peptide Modulates Ciliary Beating in the Gills of a Venerid Clam, *Mercenaria mercenaria*

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Abstract. The activities of both the lateral and frontal cilia of *Mercenaria mercenaria* were unaffected, either by the two endogenous SCP-related peptides AMSFYFPRM-amide and YFAFPRQamide, or by FMRFamide (all at 10^{-6} *M*). Dopamine (DA) inhibited the lateral cilia; the mean EC_{50} was 2×10^{-6} *M*. The peptide YFAFPRQamide—but neither AMSFYFPRMamide nor FMRFamide—antagonized the inhibition induced by DA; this effect was dependent on both time and dose. At a DA concentration of 5×10^{-7} *M*, the effect of YFAFPRQamide appeared within 20 min and became maximal within 40–60 min; the mean EC_{50} at these times was 4.7×10^{-11} *M*. If the concentration of DA was increased to 10^{-6} *M*, the maximal effect of the peptide was delayed to 50 min, and the mean EC_{50} increased to 1.1×10^{-7} *M*. Particle transport by the frontal cilia was inhibited by 5-hydroxytryptamine (5HT); the mean EC_{50} was 5.7×10^{-7} *M*. Again, only YFAFPRQ-amide had an antagonistic effect on the 5HT-induced inhibition. At a 5HT concentration of 10^{-6} *M*, the effects of YFAFPRQamide did not appear until 45 min; the mean EC_{50} was 10^{-6} *M*. When radioimmunoassayed with an SCP antiserum, the elution profile of a gill extract overlapped those of the SCP-related peptides that had previously been identified in extracts of whole animals. These data suggest that all three SCP analogs occur in the gill. Immunohistochemistry of the gill, carried out with a monoclonal antibody raised to SCP_B, stained many varicose neuronal fibers. Most of these were associated with the gill musculature, but a sparse innervation of the filaments underlying the cilia was also observed. Some fluorescent nerve cell bodies were also seen in the gill tissue. Our results are consistent with the hypothesis that YFAFPRQamide modulates branchial activities—muscular as well as ciliary—that are associated with feeding.

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Introduction

Molluscs are ciliary organisms; they are among the largest animals, yet cilia perform mechanical functions that, in many other taxa, are carried out primarily by muscles. For example, cilia are responsible for locomotion in gastropods as large as the lightning whelk *Busycon contrarium* and the helmet conch *Cassis tuberosa* (see Gainey, 1976; and Miller, 1974, respectively). Cilia are particularly well known for generating the currents that provide for respiration and feeding in all bivalves, except the Septibranchia. These currents are substantial; clearance rates generated by cilia in the American oyster *Crassostrea virginica* are as high as 24 to 27 l/h (Loosanoff and Nomeijko, 1946; Collier, 1959 [both cited in Foster-Smith, 1975]).

The ctenidial water currents are created by the lateral cilia (Purchon, 1968; Morton, 1983), although the abfrontal cilia may contribute between 30% and 40% of the flow in *Mytilus edulis* (Jones and Richards, 1993). The control of lateral ciliary activity, especially in *Mytilus*, has been studied for nearly a century (early work reviewed in Aiello, 1960; Paparo, 1972, 1985). In brief, the ciliated cells of bivalve gills are electrically coupled (Motokawa and Satir, 1975; Murakami and Machemer, 1982; Saimi *et al.*, 1983b; Stom-

mel, 1984a), and branches of the branchial nerve run beneath the lateral and frontal ciliated cells (Aiello and Guideri, 1965; Paparo, 1972; Owen, 1974; Aiello, 1979). Apparently not all of the ciliated cells are innervated, but those that do receive neural input are reported to act as pacemakers (Paparo, 1972).

Both 5-hydroxytryptamine (5HT) and dopamine (DA) have been localized in the branchial nerves of *Mytilus* (Paparo and Finch, 1972; Stefano and Aiello, 1975). Moreover, electrical stimulation of either the cerebrovisceral connective or the branchial nerve at a stimulus frequency of 10 Hz increased the rate of beat of the lateral cilia, whereas stimulation at a frequency of 20 Hz decreased the rate. These excitatory and inhibitory effects of electrical stimulation were blocked, respectively, by serotonergic and dopaminergic antagonists (Catapane *et al.*, 1978, 1979; Catapane, 1983). Applied exogenously to isolated gills, 5HT stimulates the lateral cilia of all bivalves studied to date, including those of *Mercenaria mercenaria* (see Aiello, 1962, 1970, 1990; Paparo, 1972; Motokawa and Satir, 1975; Catapane, 1983). In contrast to the effect of 5HT, the response of lateral cilia to DA is variable. For example, the lateral cilia of *Mytilus edulis, Crassostrea virginica* (Paparo and Aiello, 1970; Catapane, 1983; Paparo, 1985), *Ostrea edulis, Mercenaria mercenaria,* and *Modiolus modiolus* (Gainey and Shumway, 1991) are inhibited by DA; but the lateral cilia of *Geukensia (=Modiolus) demissa* (Catapane, 1983), *Argopecten irradians,* and *Mya arenaria* (Gainey and Shumway, 1991) are unaffected. In summary, both 5HT and DA are present in the gills of at least some bivalves, and they appear to serve as endogenous transmitters regulating, in part, the activity of the lateral cilia.

Dose-response curves for 5HT and DA (see Catapane, 1983) show that the lateral cilia of *Mytilus* have a maximal beat frequency of about 25 beats/s, and that synchronous beating is lost below about 10 beats/s. Between these narrow limits (*i.e.*, 25 and 10 beats/s), the cilia respond in a graded manner, both to stimulation by 5HT and to inhibition by DA. At the lower limit (10 beats/s), these compounds seem to be activating a simple on-off switch. That such a switch controls pumping in intact animals has yet to be demonstrated unequivocally (Stefano *et al.*, 1977; Jorgensen, 1989; Jones and Richards, 1993).

In contrast to the lateral cilia, which transport water, the frontal cilia receive material that has been retained by the branchial filter and transport it to the food grooves; there it is packaged in mucus and carried to the labial palps (Purchon, 1968; Morton, 1983; Murakami, 1989). The frontal cilia are therefore intimately involved in feeding, and their activity is correlated with the rate of mucus secretion (Aiello, 1979). Beyond that generality, the pharmacology and control of the frontal cilia is poorly understood (reviewed by Aiello, 1990).

The inconsistency between the range of clearance rates in intact bivalves and the pharmacology of isolated gill cilia, as well as the distinct functions of cilia in different tracts, suggests that ciliary activity is probably not controlled by motoneurons that release only dopamine or serotonin. Studies of a pair of neurons in the pedal ganglia of the nudibranch *Tritonia diomedea* clearly show that, in this mollusc, peptides are also involved. These neurons innervate the locomotory cilia on the foot of *Tritonia,* augment the frequency of ciliary beat when stimulated, and synthesize and store a family of three pedal peptides (Peps). Moreover, the action of these peptides mimics neuronal stimulation by increasing the frequency of ciliary beating (reviewed by Willows *et al.*, 1997). The beat frequency of vertebrate cilia, particularly those of airway epithelia, are also regulated by neuropeptides, including Substance P (Lindberg and Mercke, 1986; Lindberg *et al.*, 1986; Lindberg and Dolata, 1993; Aiello *et al.*, 1991); vasoactive intestinal polypeptide (VIP) (Lindberg *et al.*, 1988); neuropeptide Y (NPY) (Cervin *et al.*, 1991; Wong *et al.*, 1998); endothelin (Tamaoki *et al.*, 1991); and vasopressin (Tamaoki *et al.*, 1998).

Among bivalved molluscs, three members of the SCP-related family of peptides have been isolated from the quahog *Mercenaria mercenaria:* 1AMSFYFPRMamide, AMSFYFPRMamide, and YFAFPRQamide; the second peptide is likely a degradation product of the first. Furthermore, high levels of these peptides occur in the gills, and SCP-related immunoreactivity has been localized to neural fibers in the gill. But though these peptides affect gut motility in the clam (Candelario-Martinez *et al.*, 1993), their effects upon ciliary activity in the gill have not yet been tested. We have, therefore, examined two of these SCPs—YFAFPRQamide and AMSFYFPRMamide—as well as DA, 5HT, and another neuropeptide, FMRFamide, for their actions upon both the lateral and frontal gill cilia of *Mercenaria.* The results indicate that one of the peptides, YFAFPRQamide, modulates the effects of the amines. Preliminary results of this study were presented to the Society for Integrative and Comparative Biology (Gainey *et al.*, 1997).

Materials and Methods

Animals

Quahogs (*Mercenaria mercenaria* L.) were obtained from Poquoson and Wachapreague, Virginia. The animals were held at 10°C in natural seawater (30‰) on a 12 h light/dark cycle. Individuals were held a minimum of 3 days prior to use.

The preparation

Gills were dissected away from the body wall distal to the visceral ganglia and were then separated into demibranchs. The dissection caused the beating of the lateral cilia to cease

for an hour or more. But once the beating had resumed, the frequency remained unchanged for up to 24 h. Therefore, the gills were excised between 4 and 15 h before an experiment. Dorsoventral strips about 1 cm wide were cut from the isolated demibranchs and pinned to strips of rubber band that had been glued with rubber cement to the bottom of petri dishes (4.7 cm diameter). The dishes were filled with 5 ml of artificial seawater (recipe in Welsh *et al.*, 1968).

Drugs

Peptides were synthesized at the Protein Chemistry Core Facility of the Interdisciplinary Center for Biotechnology Research at the University of Florida, Gainesville. Dopamine (DA) and 5-hydroxytryptamine (5HT) were purchased from Sigma Chemical, St. Louis, Missouri.

Responses of the lateral cilia

The activity of the lateral cilia was measured as follows: Isolated, pinned-out strips of gill were placed on the stage of a compound microscope and observed at a magnification of 100×. The substage illuminator on the microscope was replaced with a mirror, and the rate of beating of the lateral cilia was determined by their synchrony with a Pasco Sf-9211 strobe light. Details of the measurement procedure are described in Gainey and Shumway (1991).

At the outset of each experiment, we would locate an area of the gill with well-defined metachronal waves and with frequencies between 12 and 25 beats/s. Once the initial rate was measured, the gill was not moved, and the same patch of cilia was used for all subsequent measurements. In all but the initial set of experiments on the effects of the peptides alone, two pieces of gill were used on separate microscopes, with one of these pieces serving as a control.

Larger quahogs (7 to 9 cm long) had lateral cilia that were consistently less sensitive to DA than those of smaller quahogs (5 to 7 cm). Moreover, the sensitivity of the lateral cilia of the smaller quahogs followed a seasonal pattern; they were less sensitive to DA from April to June. Therefore, all of the experiments reported here were done with gills from smaller animals and were carried out from June to April.

Effects of peptides: (1) Stimulation. Freshly dissected gill strips showing no lateral ciliary activity were exposed to one of the peptides at 10^{-6} *M;* controls were untreated. 5HT at 10^{-6} *M* was used as a positive control because it excites quiescent lateral cilia of *Mercenaria mercenaria* (Aiello, 1970). The rate of beat of the lateral cilia on the treated and control strips was measured hourly for 3 h. In a separate observation, we examined 10 areas on each strip of gill for the presence or absence of metachronal waves; the percentage of areas with metachronal waves (percent activity) was taken as an estimate of the ciliary activity of the strip. The data on rate and on percent activity were analyzed with ANCOVA with time as a covariate; the analysis was performed with the general linear models (GLM) procedure in SAS, version 6.

Effects of peptides: (2) Inhibition. Isolated strips of gill with active lateral cilia were exposed to one of the peptides at 10^{-6} *M;* controls were untreated. Measurements were made every 2 min for the first 10 min and then at 20, 40, and 60 min. The effects of the peptides on the rate of ciliary beating were evaluated with a two-way ANOVA with treatment, time, and treatment*time as factors; the analysis was performed with the GLM procedure in SAS.

Effects of peptides and DA. Previous studies have shown that 10^{-4} *M* DA will, within several minutes, completely arrest the lateral cilia of *Mercenaria* (Gainey and Shumway, 1991). This effect is temporary because DA slowly oxidizes, and the cilia eventually return to their initial rate of beating. To assess the effects of the peptides on this DA arrest, we exposed isolated demibranch strips to concentrations of 10^{-6} *M* of each peptide; 10 min later, the same demibranch was exposed to 10^{-4} *M* DA. Controls were exposed only to 10^{-4} *M* DA. The activity of the lateral cilia was recorded every 2 min until it returned to the initial rate. In some instances, the ciliary beating on one of the gill strips did not return to its initial rate before the end of the experiment; data of this type were designated *censored.* The results were analyzed with the Wilcoxon test with the lifetest procedure in SAS; this program adjusts for censored data.

Dose-dependent effects. In these experiments, two strips taken from the same demibranch were pinned out and observed with separate microscopes. The initial rate of ciliary beating of both strips was then determined. Thereafter, the measurement of ciliary activity at any time was expressed as a fraction of the initial rate; *i.e.,* the *fractional rate* of beat. After a drug of interest was applied, the fractional rate of the treated strip was corrected by subtraction of the fractional rate of the control strip. This *fractional difference* was taken as the measure of peptide effect and was used as the ordinate on dose-response curves.

(1) *Dopamine.* In these experiments, oxidation of DA was retarded with an ascorbic acid buffer as described by Malanga (1975a). DA was added to the treated strip; the control strip was untreated; and the rate of beat of both strips was determined every 10 min for 1 h. Each pair of gill strips was used to measure only one dose of DA. Because DA is inhibitory, the value of the fractional difference becomes larger and more negative with dose. Therefore, to make the DA dose-response plots more comprehensible, the effect was expressed as the *adjusted fractional difference:* $[1 + (\text{fractional rate}_{\text{treated}} - \text{fractional rate}_{\text{control}})]$.

(2) *YFAFPRQamide.* After the measurement of initial rate, a dose of peptide was added to the treatment strip. Ten minutes later, DA (either 5×10^{-7} *M* or 10^{-6} *M*) was added to both the treatment and control gills. Thereafter, rates

were measured every 10 min for 1 h. The fractional difference was used as the measure of effect in the dose-response curves; notice that when the response of peptide is maximal, no inhibition by DA is observable, so the fractional rate of the control strip approximates zero.

Responses of the frontal cilia

The activity of the frontal cilia was measured as follows: Isolated, pinned-out strips of gill were observed at a magnification of 100× with a compound microscope, and the activity of the frontal cilia was determined by the rate of transport of polystyrene microspheres (diameter, 0.85–1.0 μm; Polysciences, Inc., Warrington, Pennsylvania). The time (in seconds) required for these particles to travel 0.5 mm was measured with a stopwatch and an ocular micrometer. Particle transport rates (mm/s) were expressed as a fraction of the initial rate. During the experiments on peptides, five readings were taken on each gill strip at each time. But during the experiments on the effects of the peptides and 5HT, three readings were made at each time. Once the initial rate was measured, the gill strips were not moved, and the same gill filaments were used for all subsequent measurements.

Effects of peptides. Isolated strips of gill were exposed to one of the peptides at 10^{-6} *M;* controls were untreated. Particle transport rates were measured every 5 min for 25 min. Initial analysis of these data indicated a positive correlation between the standard deviation and the mean of the fractional initial rate. Therefore, the data were transformed with natural logarithms, which removed this correlation. The effects of the peptides on the frontal cilia, as well as the effects of the peptides plus 5HT, were evaluated using repeated measures ANOVA; the analyses were performed using the GLM procedure in SAS.

Effects of peptides with 5-hydroxytryptamine. We found that 5HT inhibits the rate of particle transport by the frontal cilia. To assess the effects of the SCPs on this inhibition, we first exposed isolated strips of gill to the SCPs at 10^{-6} *M;* 10 min later, the same gill strip was exposed to 10^{-6} *M* 5HT. Control strips were exposed only to 10^{-6} *M* 5HT. Particle transport rates were measured every 15 min for 1 h.

Dose-dependent effects. We followed almost the same protocols and analyses that were used to examine the effects of DA and of YFAFPRQamide plus DA on the lateral cilia. The exceptions were that (1) readings were taken every 15 min for 1 h; and (2) three replicate readings on each gill strip at each time were averaged, and the average rates were expressed as a fraction of the average initial rate of each strip. In the experiments on the effects of YFAFPRQamide plus 5HT, the concentration of 5HT was 10^{-6} *M.*

Regression analyses, significance levels

All dose-response curves, and the concentrations of agonists giving half-maximal responses (EC_{50}), were estimated, at each time, from a logistic model [response = $1/(1+e^{(\beta 0+\beta 1*\log\ dose)})$], with a nonlinear regression procedure (Nlin) in SAS. *F* tests were used to compare the regression lines using a general linear test approach (Bates and Watts, 1988; Neter *et al.,* 1990). In most instances, means are reported with their standard errors and sample sizes. All test statistics, including ANCOVA and ANOVAs, were considered significant at probabilities less than 0.05.

Detection of SCPs in clam gill

Radioimmunoassay of a fractionated extract. In 1993, Candelario-Martinez *et al.* tabulated the distribution of SCP-related immunoreactivity among the tissues of *M. mercenaria* (see their table 1). In this paper, we present the unpublished immunoreactive profile of the SCPs in gill, which were obtained as follows.

Gills from 20 animals were extracted in acetone. The extract was evaporated, and the aqueous portion was loaded onto a Prep-10 Octyl column (10 × 100 mm, 4 ml/min) and eluted with a gradient of acetonitrile (16%–40% over 30 min) in water with 0.1% trifluoroacetic acid. Fractions were collected every half minute and analyzed by radioimmunoassay; elution patterns were plotted from these data. Details of the fractionation and the assay are set out in Candelario-Martinez *et al.* (1993).

Immunohistochemistry. Small, rectangular pieces of tissue were cut from the outer demibranchs of several clams; the samples usually included the ventral edge of the gill and were 2–3 mm wide and 3–5 mm high. A few minutes after dissection, the tissues were fixed in a solution of paraformaldehyde, prepared freshly as follows. A solution of paraformaldehyde (4 g in 45 ml distilled water) was heated at 60°C for 10 min, clarified by the addition of 1 *N* NaOH, brought to a final volume of 50 ml, and cooled on ice for about 20 min. Thereafter, 50 ml of 0.2 *M* sodium potassium phosphate buffer (SPB) was added, together with 15 g of sucrose to approximate the osmolality of seawater. The tissues were left in this fixative overnight at 4°C.

After fixation, the tissues were rinsed twice (5 min each) with Tris buffered saline (TBS; pH 7.4), and then placed in 30% sucrose/PBS and left overnight at 4°C. The tissues were then embedded in Tissue Tek O.C.T., frozen, and sectioned (10 μm). The sections were collected onto gelatin-coated slides, and stored at −80°C for at least 24 h prior to staining.

The sections were preincubated for 30 min at 37°C in TBS containing 0.1% Triton X-100 and 2% normal goat serum. The preincubation medium was then poured off and replaced with the primary antibody—a monoclonal raised to SCP_B (Masinovsky *et al.,* 1988)—which was diluted 1:100

in the preincubation medium. After 4 h in the primary antibody at room temperature, the sections were rinsed three times (5 min each) in TBS, and secondary antibody—fluoresceine isothiocyanate-conjugated goat anti-mouse IgG (FITC-GAM IgG)—was then applied to the sections; incubation continued for 2 h, at room temperature, in the dark. The sections were then washed once for 5 min in TBS containing 10 μg/ml of 4′,6-diamidino-2-phenylindole (DAPI), an ultraviolet-excitable, nucleic acid-binding dye. The sections were washed twice more (5 min each) in TBS, and coverslips were applied; the mounting medium was 60% glycerol/TBS containing *p*-phenylenediamine (PPD). Controls were treated as described above, except that the primary antibody, before being applied to the sections, was incubated overnight, at 4°C, on a rotating shaker, with either YFAFPRQamide or AMSFYFPRMamide (10^{-3} *M*).

Micrographs were generated with a Leica/Leitz DMRB microscope equipped with filters that allow the mutually exclusive visualization of fluorochrome and DAPI staining. Digital images were gathered with a Hamamatsu color chilled 3CCD camera (C5810) and were prepared for printing with Adobe Photoshop.

Results

Lateral cilia

Peptides. Analysis of preliminary experiments on gill strips exposed to either AMSFYFPRMamide, YFAFPRQamide, or FMRFamide (all at 10^{-6} *M*) revealed that none of the peptides had any significant stimulatory or inhibitory effect upon the activity of the lateral cilia: stimulation (ciliary rate), $F_{(3,119)} = 0.85$, $P = 0.47$; stimulation (percent activity), $F_{(3,79)} = 0.37$, $P = 0.78$ (Table I); inhibition (ciliary rate), $F_{(3,28)} = 0.26$, $P = 0.85$. In the experiment on the inhibition of spontaneous ciliary activity, the mean rate of beat of the control cilia, as well as those treated with any of the three peptides, was 25 beats/s ($n = 9$ gills for each treatment).

Table I

Responses of quiescent lateral cilia exposed to peptides and 5HT at 10^{-6} M

Treatment	Frequency (beats/s)	SE	% activity	SE	*n*
Control	11	1.1	36	5.5	6
AMSFYFPRMa	12	1.1	31	5.5	6
YFAFPRQa	10	1.1	30	5.5	6
FMRFamide	12	1.1	30	5.5	6
5HT*	12	1.1	57**	5.8	6

Controls were untreated; *n* = number of gill-strip preparations. Responses are frequency (beats/s); or as the percent occurrence of metachronal waves in 10 separate areas of each gill (% activity). The data are all expressed as least square means.

* Excluded from the ANCOVA in text.

** Significantly greater ($P < 0.05$) than the control and the peptides.

Table II

Comparison of mean times to recovery (±SE) for lateral cilia exposed simultaneously to peptides (10^{-6} M) *and DA* (10^{-4} M), *and for lateral cilia exposed only to DA* (10^{-4} M)

	Recovery time			
Treatment	Treated (min)	Control (min)	*n*	*P***
AMSFYFPRMa	62 ± 9.26	60 ± 9.44	5	0.60
YFAFPRQa	43 ± 9.04	64 ± 8.19	6	0.02
FMRFamide	69 ± 9.16	62 ± 5.36	6	0.93

n = number of gill strip preparations.

** *P* values were generated with a Wilcoxan test.

Peptides and dopamine. To determine whether the peptides might alter the activity of lateral cilia inhibited by DA, we exposed isolated gill strips to individual peptides at 10^{-6} *M*, and 10 min later to 10^{-4} *M* DA. Control strips were exposed only to DA. An ascorbic acid buffer was omitted in these experiments, so DA oxidized and the ciliary beat recovered. The recovery times of lateral cilia exposed, at first, to either AMSFYFPRMamide or FMRFamide, and then to DA, were not significantly different from those of the controls (DA only, Table II). In contrast, the lateral cilia of gill strips exposed to YFAFPRQamide and then to DA returned to their initial rates within 42 ± 9.0 min, whereas the DA controls required 64 ± 8.2 min to return to their initial rates; these times are significantly different (Table II).

Dose-dependent effects. The adjusted fractional differences, measured at several times, were plotted against the log of the DA concentration, and the family of calculated regression lines is set out in Figure 1A. This graph shows that the effects of DA appear within 10 min of the treatment and remain constant for 1 h; there is no statistical difference among the regression lines plotted in Figure 1A ($F_{(10,120)} = 0.22$, $P = 0.99$). The mean EC_{50}, as estimated from the regression parameters, is 2.0×10^{-6} *M* ($\pm 6.9 \times 10^{-8}$ *M*). But the dose-response curves are very steep; the change from 90% to 10% maximal activity is effected by an increase of only half a log unit in the concentration of DA (3–4 μ*M*). Moreover, Figure 1B shows that the response is essentially biphasic; *i.e.*, the cilia are either beating or not at a DA concentration of about 3 μ*M*.

The dose-dependent effects of YFAFPRQamide on DA-treated cilia were studied on gills exposed to 5×10^{-7} *M* DA. This concentration of the amine was chosen because it was predicted (from the dose-response regression equation) to inhibit the cilia by 17% of their initial rate; thus the peptide could, in theory, either potentiate or inhibit the effects of DA. The actual response of the control gills to DA was quite variable, ranging from 10% to 100% inhibition of the original rate, but YFAFPRQamide always had an

antagonistic effect on the action of DA. That is, gill strips treated with varying concentrations of the peptide and 5×10^{-7} *M* DA were inhibited less than gill strips exposed to DA alone. The threshold for the effect of YFAFPRQamide was about 5×10^{-12} *M*. The maximal response (*i.e.*, complete block of inhibition) was produced by about 10^{-8} *M*. Moreover, the antagonistic effects of the peptide were time dependent. A set of dose-response regression lines produced at 10-min intervals shows that the effects of the peptide began to appear within 20 min after the addition of DA. The regression lines from 40 to 60 min are not statistically different ($F_{(4,44)} = 0.71$, $P > 0.05$; Fig. 2A).

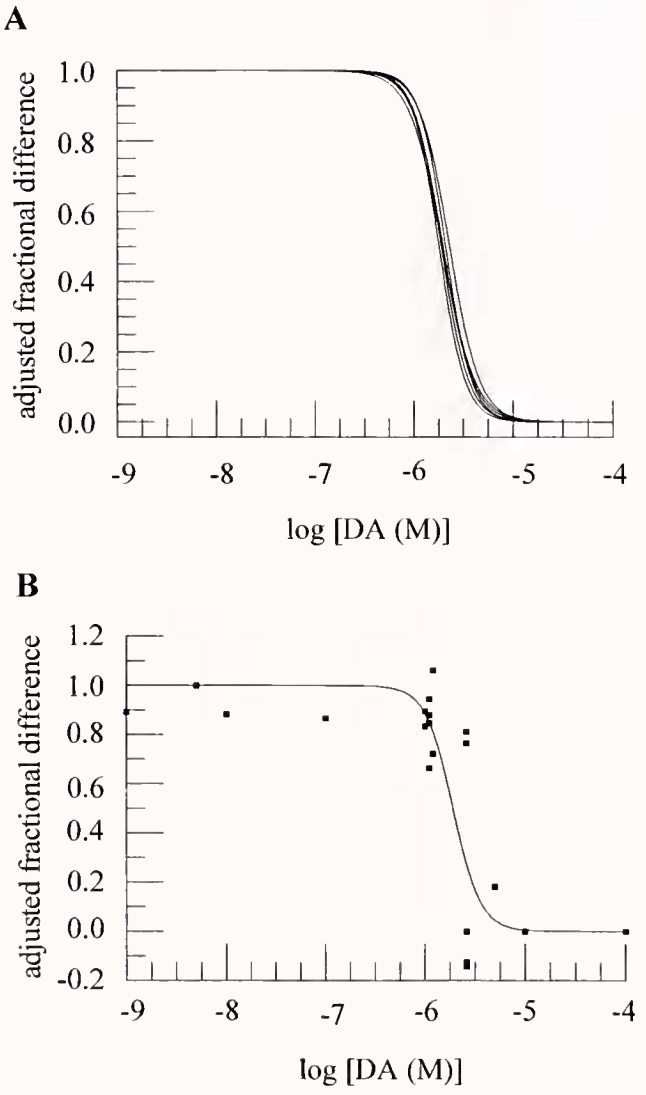

Figure 1. (A) Dose-response curves for the inhibitory effect of dopamine (DA) on lateral cilia. Ordinate: the *adjusted fractional difference*: [1+ (fractional treatment rate − fractional control rate)]; this measure is described in the Methods section. The regression lines were derived from measurements made every 10 min after the addition of DA, continuing to 60 min. Data points are omitted for clarity. (B) The regression line at 60 min with data points shown.

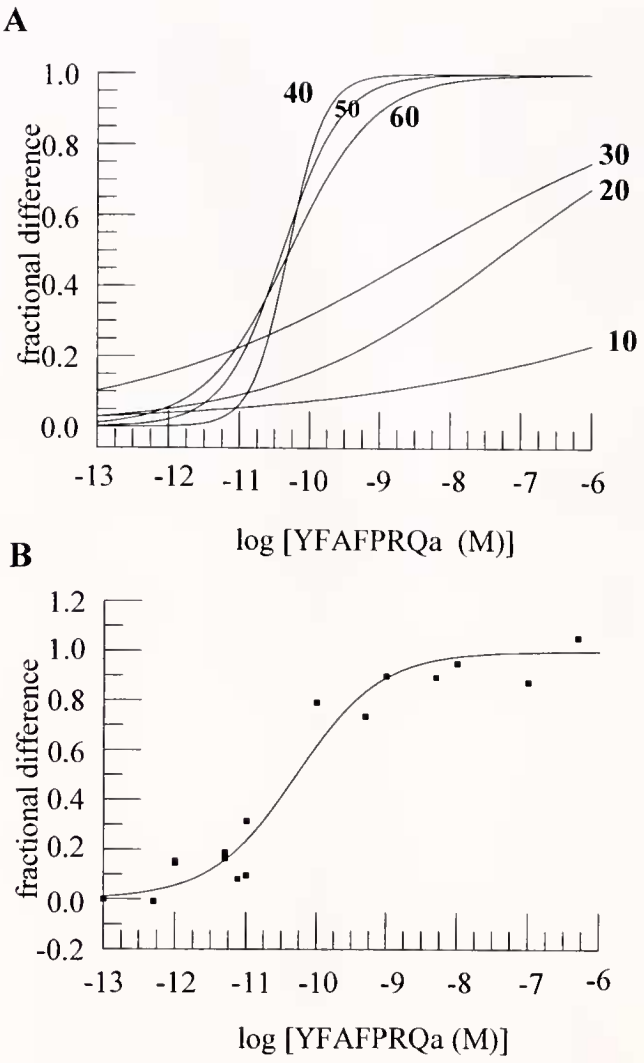

Figure 2. (A) Dose-response curves illustrating the antagonistic effect of YFAFPRQamide on the dopamine- (DA-) induced inhibition of lateral cilia. Ordinate: the *fractional difference* between lateral cilia exposed to 5×10^{-7} *M* DA plus various concentrations of YFAFPRQamide, and lateral cilia exposed only to 5×10^{-7} *M* DA. Peptides were added 10 min before the cilia were exposed to DA. The regression lines were generated at the times indicated *after the addition of DA*. The slope parameter ($\beta 1$) of the line at 10 min is not significantly different from 0. Data points are omitted for clarity. (B) The regression line at 60 min with data points included.

The mean EC_{50} for these times is 4.7×10^{-11} *M* ($\pm 3.5 \times 10^{-12}$ *M*).

To determine whether the latency in the effect of YFAFPRQamide might reflect the permeability of the gills to the peptide, the concentration of DA was increased, from 5×10^{-7} *M*, to 10^{-6} *M*. If the latency were due primarily to the low permeability of the peptide, then increasing the dose of DA should only increase the EC_{50} of YFAFPRQamide and not alter the time course of the response. In fact, the threshold for the effect of the peptide increased from

5×10^{-12} *M* to about 10^{-8} *M;* the dose producing the maximal effect increased from 10^{-8} *M* to about 5×10^{-7} *M*. Again, the antagonistic effects of the peptide on the DA-induced inhibition were time dependent, and the effect began to appear 20 min after the addition of DA. But, the response did not stabilize until 50 min after the addition of dopamine (Fig. 3A), as opposed to 40 min previously (compare with Fig. 2A). Moreover, the 40-min regression lines for the two doses of DA are significantly different (compare Figs. 2A and 3A; $F_{(2,28)} = 10.3$; $P = 0.0004$). And, the regression lines at 50 and 60 min in Figure 3A are not statistically different ($F_{(2,26)} = 0.45$, $P = 0.64$); the mean EC_{50} for these times is 1.1×10^{-7} *M* ($\pm 2.6 \times 10^{-8}$ *M*). Finally, a Mann-Whitney *U* test revealed that the mean EC_{50} of gills exposed to YFAFPRQamide and 10^{-6} *M* DA was significantly greater than that of gills exposed to YFAFPRQamide and 5×10^{-7} *M* DA ($P = 0.04$). Thus, the latency of the peptide response cannot be due entirely to permeability.

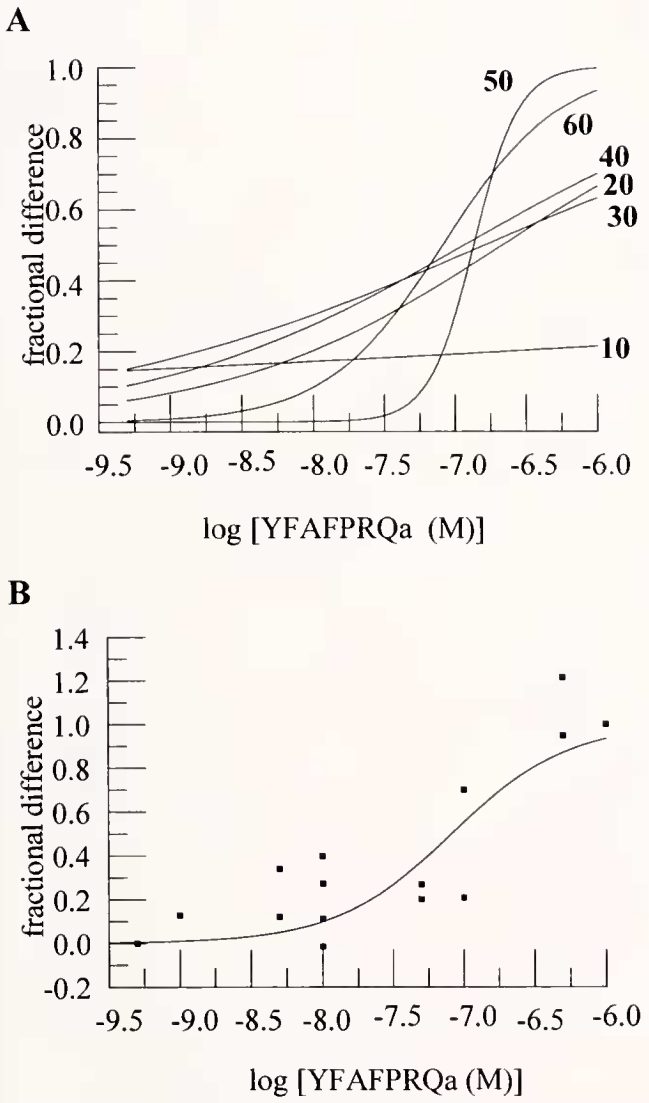

Figure 3. (A) Effect of a higher dose of dopamine (DA) on the dose-response curves for the antagonistic effect of YFAFPRQamide (compare with Fig. 2). Ordinate: the *fractional difference* between lateral cilia exposed to 10^{-6} *M* DA plus various concentrations of YFAFPRQamide, and lateral cilia exposed only to 10^{-6} *M* DA. Peptides were added 10 min before the cilia were exposed to DA. The regression lines were generated at the times indicated after the addition of DA. The slope parameter ($\beta 1$) of the line at 10 min is not significantly different from 0. Data points are omitted for clarity. (B) The regression line at 60 min with data points included.

Beat frequencies. The lateral cilia used in dose-response studies, including controls, beat in metachronal waves at frequencies from 7 to 27 beats/s, or they did not beat at all. That is, no metachronal waves appeared at frequencies lower than 7 beats/s ($n = 888$ on 148 pieces of gill).

Frontal cilia

Peptides. Preliminary experiments on gill strips exposed to the three peptides, all at 10^{-6} *M*, revealed that none of the peptides had a significant effect on the activity of the frontal cilia: AMSFYFPRMamide, $F_{(1,78)} = 0.05$, $P = 0.81$; YFAFPRQamide, $F_{(1,78)} = 1.66$, $P = 0.20$; FMRFamide, $F_{(1,48)} = 3.18$, $P = 0.08$; the mean rate of particle transport in all cases was 0.29 mm/s.

SCPs and 5-hydroxytryptamine. To determine whether the two SCPs might alter the activity of frontal cilia inhibited by 10^{-6} *M* 5HT, we first exposed isolated gill strips to either AMSFYFPRMamide or YFAFPRQamide (10^{-6} *M*) and then, 10 min later, to 10^{-6} *M* 5HT. Controls were exposed only to 5HT. Neither peptide had a significant effect upon the 5HT-induced inhibition: AMSFYFPRMamide, $F_{(1,16)} = 1.22$, $P = 0.28$; YFAFPRQamide, $F_{(1,8)} = 3.93$, $P = 0.08$.

Dose-dependent effects. In Figure 4A, the regression lines of the adjusted fractional difference are plotted against the log of the 5HT concentration and time. The graph shows that the inhibitory effects of 5HT on the frontal cilia appear within 15 min and remain constant for 1 h; there is no statistical difference between these regression lines ($F_{(6,34)} = 0.32$, $P = 0.92$). The mean EC_{50} is 5.7×10^{-7} *M* ($\pm 3.5 \times 10^{-7}$ *M*). Particle transport was, however, never completely inhibited; the maximal inhibition was about 80% of the initial rate at 10^{-3} *M* 5HT.

The effect of YFAFPRQamide on frontal cilia inhibited by 5HT was not statistically significant. But because the probability of the "*F*" value from the ANOVA was 0.08, which is close to the significance level of 0.05, and because the peptide had modulated the action of DA on lateral cilia, we decided to measure the dose-dependency of the effects of the peptide on 5HT-induced inhibition. The frontal cilia were exposed to 10^{-6} *M* 5HT, a concentration predicted to inhibit the cilia by 39% of their initial rates, and to varying concentrations of the peptide. The threshold for the effect of

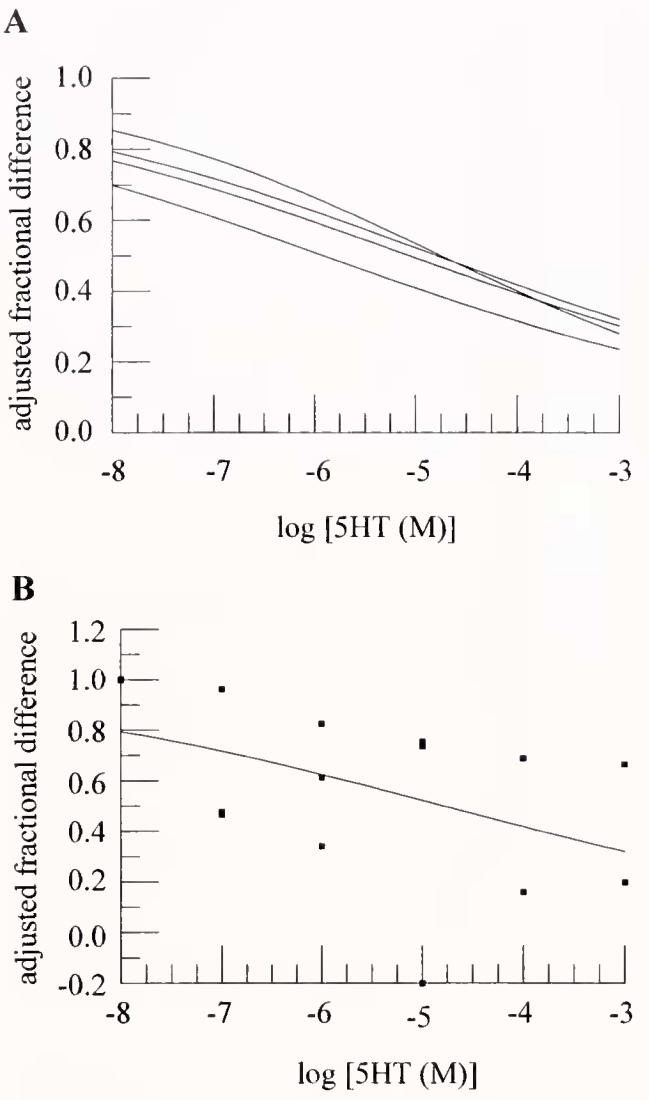

Figure 4. (A) Dose-response curves for the inhibitory effect of 5-hydroxytryptamine (5HT) on frontal cilia. Ordinate: the *adjusted fractional difference:* [1+ (fractional treatment rate − fractional control rate)]; explained in the Methods section. The regression lines were derived from measurements made every 10 min after the addition of 5HT, continuing to 60 min. Data points are omitted for clarity. (B) The regression line at 60 min with data points included.

YFAFPRQamide was about 5×10^{-7} *M*. At the maximum dose of YFAFPRQamide used (3×10^{-6} *M*), there was about a 30% difference between the controls and the treatment cilia. This means that the cilia returned to their original rates because the 5HT did not completely inhibit them. The effects of the peptide did not appear until 45 min after the addition of 5HT, and there was no statistical difference between the regression lines at 45 and 60 min ($F_{(3,32)} = 0.1$, $P = 0.91$; Fig 5A). The mean EC_{50} is 10^{-6} *M* ($\pm 1.5 \times 10^{-7}$).

Particle transport rates. Particle transport rates of both treated and control cilia varied from 0.05 to 0.56 mm/s ($n =$ 1560 on 52 pieces of gill), but the rates were never zero in any of the experiments.

Identification of SCPs in clam gill

Radioimmunoassay. The purification of SCPs from clam extracts is strongly influenced by two features of the longest SCP, 1AMSFYFPRMamide: First, even as the purification proceeds, this peptide is degrading by stepwise cleavage at

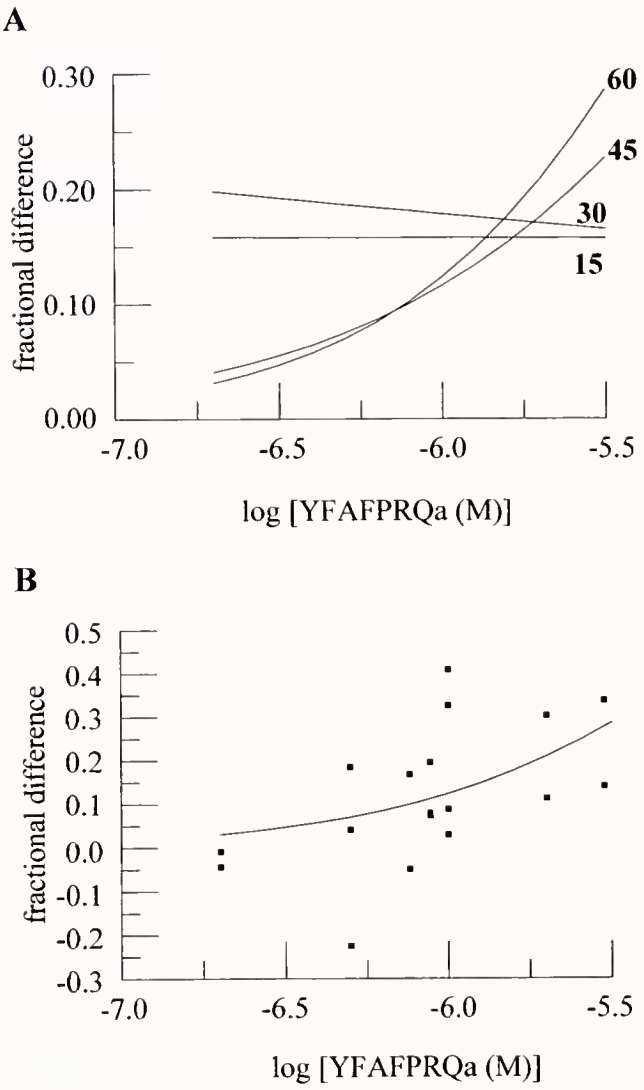

Figure 5. A) Dose-response curves illustrating the antagonistic effect of YFAFPRQamide on the 5-hydroxytryptamine- (5HT-) induced inhibition of frontal cilia. Ordinate: the *fractional difference* between frontal cilia exposed to 10^{-6} *M* 5HT plus various concentrations of YFAFPRQamide, and frontal cilia exposed only to 10^{-6} *M* 5HT. Peptides were added 10 min prior to exposure of the cilia to 5HT. The regression lines were generated at the times indicated *after the addition of 5HT.* The slope parameter ($\beta 1$) of the lines at 15 and 30 min are not significantly different from 0. Data points are omitted for clarity. (B) The regression line at 60 min with data points included.

the N-terminal. Second, the peptide and its degradation products oxidize at one or both methionine residues. Thus, peaks of immunoreactivity tend to be wide, and their position tends to shift from step to step of the purification. Nevertheless, SCP-related peptides were isolated and identified, either by mass spectroscopy or chemical sequencing, from extracts of whole clam (Candelario-Martinez *et al.*, 1993). This work is summarized in Figure 6A, which shows that, although YFAFPRQamide occurred only in fraction 10, IAMSFYFPRMamide and its oxidized forms were identified from fractions 18 to 27. The truncated peptide AMSFYFPRMamide and its oxidized forms were distributed from fraction 14 to 27. The smaller degradation products of IAMSFYFPRMamide all eluted early.

When extracts of gill were fractionated on the same HPLC system, most of the immunoreactivity eluted between 5 and 15 min (fractions 10–30). The elution profiles generated from three extracts were all very similar; *i.e.*, three peaks centered, respectively, at fractions 11, 18, and 24 (*e.g.*, Fig. 6B). No SCPs have been successfully purified from extracts of clam gills, but the near congruence of the elution profiles of extracts of gill and whole clams suggests that all of the substances found in whole clams are also present in gills. Moreover, a search for the major products (YFAFPRQamide, AMSFYFPRMamide, and IAMSFYFPRMamide) would probably begin with an examination of the three obvious peaks seen in the gill extract.

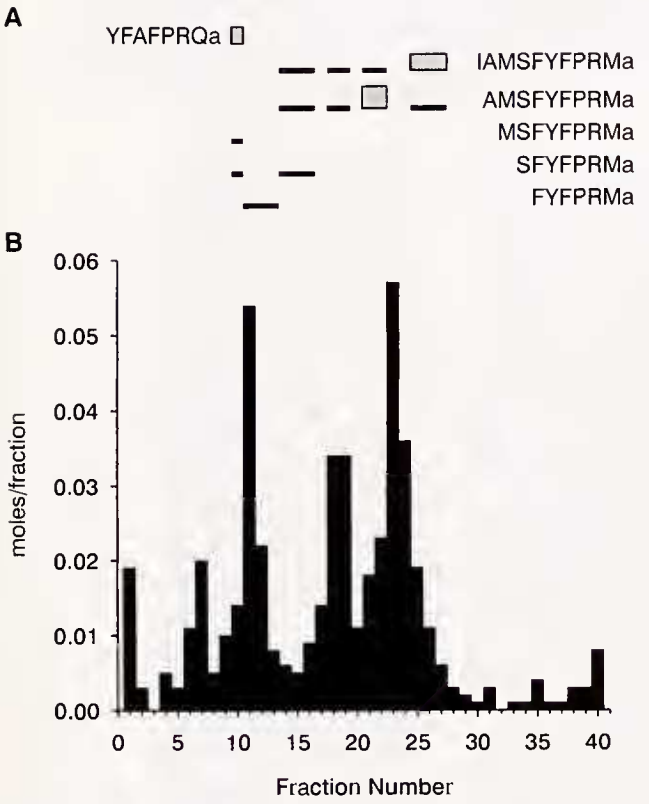

Figure 6. An assessment of the distribution of SCP-related peptides in acetone extracts of tissues of *Mercenaria mercenaria*. (A) SCP-related peptides identified in extracts of whole clams (replotted data from Candelario-Martinez *et al.*, 1993). The extract was fractionated on HPLC, as described in the Methods section. Five peaks were selected for purification, and the labeled peptides were identified, either by sequencing (grey boxes) or by FAB-ms (thickened lines). Note that IAMSFYFPRMamide, AMSFYFPRMamide, and their presumed degradation products are widely distributed in the whole clam extract, whereas YFAFPRQamide is restricted to fraction 10. (B) Elution profile of an extract of gills for comparison with the peptide distribution in A.

Immunocytochemistry. SCP-like immunoreactive staining in the gill tissue was apparent only in the fibers and cell bodies of neurons. Most of the fibers seen were varicose and occurred in the interior of the gill, in the interlamellar septa, and associated with muscle (Fig. 7A). But a very few, fine immunoreactive fibers were also observed in the proximal walls of the filament projecting toward the ciliary tracts (Fig. 7B, C). Infrequently, these fibers could be followed to the distal end of the filament, and to the base of the epithelial cells bearing the frontal cilia (Fig. 7D). Neuronal cell bodies were also stained; they appeared interior to the bands of horizontal muscle underlying the filaments (Fig. 8A). Under UV illumination, the nuclei of these cells, which were stained with DAPI, were clearly visible (Fig. 8B).

When we preabsorbed the primary SCP antibody with either YFAFPRQamide or AMSFYFPRMamide, staining was abolished (not shown). In addition, preabsorption of this monoclonal antibody with FMRFamide affected neither its immunostaining of *Tritonia diomedia* ganglia (Masinovski *et al.*, 1988) nor that of crustacean neurons (Arbiser and Beltz, 1991). However, the immunostaining is reduced or abolished upon preabsorption with TNRNFLRFamide (Arbiser and Beltz, 1991), a native peptide discovered in the lobster *Homarus americanus* (Trimmer *et al.*, 1987). On the other hand, no N-terminally extended FLRFamide analogs could be detected by a specific immunochemical analysis of HPLC fractions of ganglion extracts from *M. mercenaria* (K. E. Doble, D. A. Price, and M. J. Greenberg, unpublished results).

Discussion

We have shown that the activity of the lateral and frontal cilia of the *Mercenaria* gill are modulated specifically by low concentrations of an endogenous SCP-related neuropeptide, YFAFPRQamide. We suppose that YFAFPRQamide is a modulator because its effect—a diminution of inhibition—is apparent only on lateral and frontal cilia that have been treated with their inhibitory transmitters (DA and 5HT, respectively); *i.e.*, none of the peptides had any direct effect upon the activity of untreated, spontaneously beating cilia. Finally, the specificity of the modulation is suggested by the lack of activity, not only of FMRFamide, but also of AMSFYFPRMamide, the other SCP-related peptide tested.

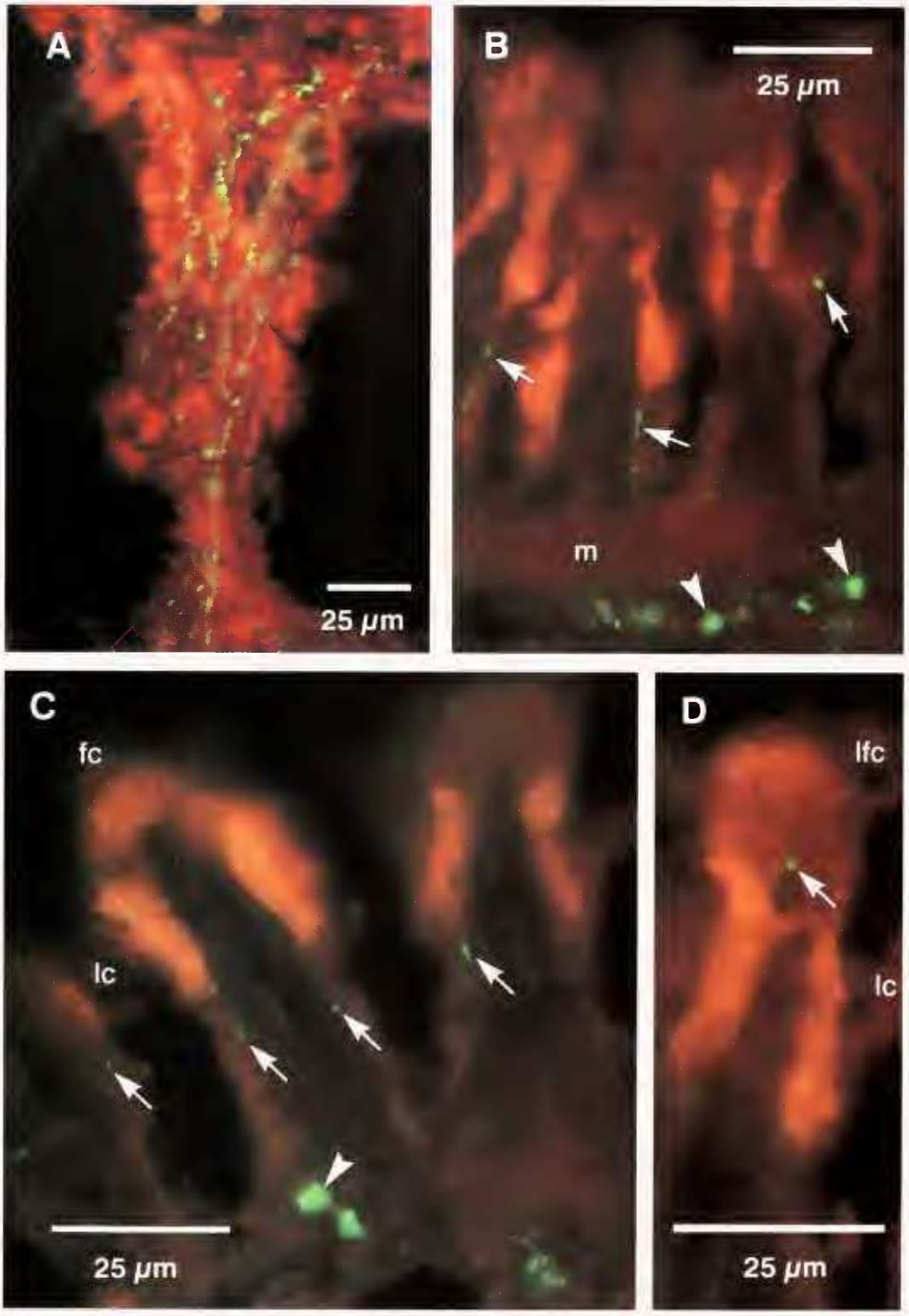

Figure 7. Frontal-sections through the gill of *Mercenaria mercenaria*. (A) SCP-related immunoreactivity occurs within varicose neural fibers in the interlamellar septa. (B–D) Innervation of the gill filaments that bear the ciliary tracts. Thin, sparse fibers (small arrows) can be followed from the base of the filament (B), to the level of the lateral cilia (C), and occasionally to the distal end of the filament below the frontal and laterofrontal cilia (D). Cell bodies (large arrowheads) are usually (*e.g.*, B), but not always (C), found proximal to the filaments. Abbreviations: fc, frontal cilia; lc, lateral cilia; lfc, laterofrontal cilia.

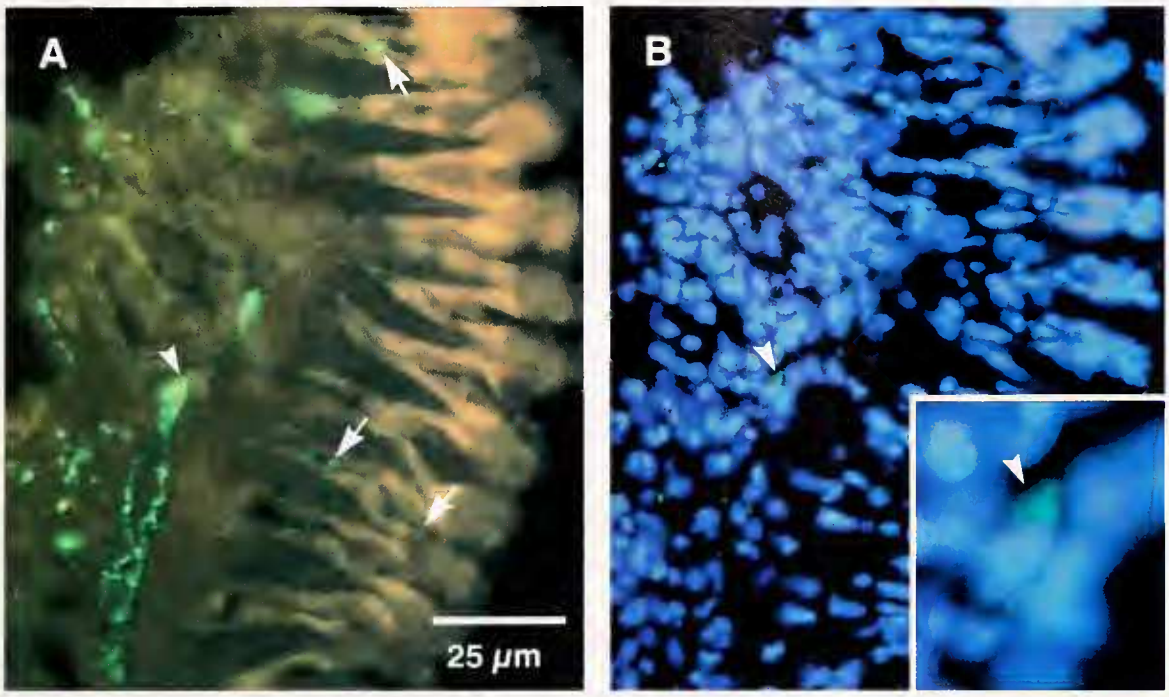

Figure 8. Neuronal cell bodies stained with the primary SCP-antibody are apparent in the gill tissue (A, arrowhead). Under UV illumination, the nucleus of such putative cell bodies can be seen to be stained with DAPI (B). The small arrows indicate fine fibers that innervate the gill filaments (see also Fig. 7).

That YFAFPRQamide and AMSFYFPRMamide (as well as the untruncated analog 1AMSFYFPRMamide) are endogenous peptides of *Mercenaria mercenaria* was clearly demonstrated by Candalario-Martinez *et al.* (1993). More to the point, however, the immunoreactive elution profile seen when gill extracts are fractionated on HPLC, taken together with our immunocytochemical observations, is consistent with the hypothesis that these peptides occur in the gill, and specifically within neurons. Furthermore, our demonstration of immunoreactive neuronal cell bodies in the gill suggests that some of the SCP-related peptides are actually synthesized within this tissue. We note, however, that the individual branchial neuropeptides have yet to be unambiguously identified in gill, and that the organization of the precursor RNA and its pattern of expression remains completely unresolved.

Although most of the immunostained SCPergic nerves are associated with muscle, particularly in the interlamellar septa, some of these fibers project out along the filament toward the lateral cilia. At the distal end of the filament, fibers appear to run along the dorsoventral axis of the filament, under the frontal and laterofrontal cilia. That these fibers actually innervate ciliated cells (and not, for example, mucus glands), and whether their origin is in the visceral ganglion or the gill, remains to be established. Nerves in the same locations have been reported in the gill filaments of *Mytilus edulis* (references in Introduction), and also in *Chlamys varia* and *Ostrea edulis* (see Owen and McCrae, 1976). Moreover, Setna (1930) and Elsey (1935) observed nerve cell bodies in, respectively, the gills of *Pecten maximus* and *Ostrea (=Crassostrea) gigas.* But all of these animals are in the subclass Pteriomorphia, and mussels and scallops possess filibranch gills. Nerve fibers have been observed, from time to time, in the eulamellibranch gills of heterodonts like *Mercenaria mercenaria, (e.g., Ensis siliqua* in Atkins, 1937), but the *innervation* of such gills has not been described. So broad generalizations about branchial innervation in the Bivalvia must be made cautiously, at present.

The effects, reported here, of endogenous YFAFPRQamide on the branchial cilia of *Mercenaria* are, in their general characteristics, reminiscent of those of VIP on the cilia of the intact rabbit maxillary sinus (Lindberg *et al.*, 1988). Immunoreactive VIP occurs in neural fibers under the epithelium, as well as in the sphenopalatine ganglion. Moreover, although arterial infusion of the peptide alone is without effect on mucociliary activity, VIP does potentiate the stimulatory action of infused methacholine. In contrast, the other peptides affecting mammalian airway cilia have observable effects on intact tissues (references in Introduction)—as do the endogenous pedal peptides (T-Peps) on the pedal cilia of *Tritonia* (see Willows *et al.*, 1997).

The T-Peps and the SCP-related peptides of *Mercenaria* also differ in the specificity of their actions. That is, only one of the bivalve homologs (YFAFPRQamide) is a ciliary modulator, whereas all three *Tritonia* homologs are roughly

equipotent in their direct stimulation of cilia. Nevertheless, an ortholog of the T-Peps—an A-Pep isolated from *Aplysia californica* (Lloyd *et al.*, 1996)—is also ineffective on the pedal cilia of *Tritonia* (Willows *et al.*, 1997).

All but one of the amino acid residues in YFAFPRQamide are either highly conserved or identical to those in the 13 known molluscan SCPs (see table II in Candelario-Martinez *et al.*, 1993). The exception is the C-terminal glutaminyl amide, and so the specific modulatory action of YFAFPRQamide on the clam gill cilia can probably be attributed to this residue. Since YFAFPRQamide is consistently more potent than AMSFYFPRMamide on the contractility and rhythmicity of the various parts of the clam gut (Candelario-Martinez *et al.*, 1993), we might suggest that the C-terminal Gln-NH_2 improves binding to a particular SCP receptor in the branchial epithelium of *Mercenaria*, as for example, the action of substance P on the ciliated epithelium of the rabbit maxillary sinus seems to be specifically at the NK1 receptor (Lindberg and Dolata, 1993). However, the rapid degradation of IAMSFYFPRMamide and AMSFYFPRMamide, relative to YFAFPRQamide, in extracts and presumably in the intact tissue, might also explain the differences in potency. If this were the case, the prolonged onset of the YFAFPRQamide modulation might be telling us that AMSFYFPRMamide is ineffective because a threshold concentration is never reached. We note that the stimulatory effects of substance P on the ciliary beat of cultured, brushed human nasal epithelial cells were also dependent upon endopeptidease activity (Smith *et al.*, 1996).

In any event, the slow development of the YFAFPRQamide effect is striking. The modulation did not appear until 20 min after treatment of the lateral cilia with DA. Moreover, when the concentration of DA was increased from 5×10^{-7} *M* to 10^{-6} *M*, the maximal effect of the SCP was delayed further, from 40 min to 50 min, and this increase was statistically significant. In comparison, the latencies of various neuropeptide effects on other molluscan tissues, though varied, are shorter. For some examples, the effects of FMRFamide on neurons of the snail *Helix aspersa* (Green *et al.*, 1994), of pedal peptides on the cilia of *Tritonia* (A. O. D. Willows, Univ. of Washington, pers. comm.), and of YFAFPRQamide on the rectum of *Mercenaria* (Candelario-Martinez *et al.*, 1993) are all evident in less than a minute. A myomodulin-related peptide (MMc) required 3 to 4 min to fully activate an L-type calcium current in the ARC muscle of *Aplysia californica* (Brezina *et al.*, 1995). The peptide achatin I maximally reduced the inward current in neurons of the snail *Achatina fulica* in about 15 min (Liu and Takeuchi, 1995). Finally, SCP_B took up to 30 min to maximize adenylate cyclase activity in ganglionic homogenates of the snail *Planorbis corneus* (Ferretti *et al.*, 1996). Thus, the time required for YFAFPRQamide to exert its full effect on the lateral and frontal cilia of *Mercenaria* is the longest reported in molluscs.

Because the inhibitory effect of DA develops much more rapidly than that of YFAFPRQamide, and the molecular weight of DA (189.6 Da) is less than that of YFAFPRQamide (927.44 Da), the slow onset of the peptide action might reflect the inverse proportionality between the diffusion coefficient of a molecule and the square root or cube root of its molecular weight (Alberty, 1983; Denny, 1993). Thus, the ratio of the diffusion coefficients of YFAFPRQamide and DA should be between 0.47 and 0.59. But pyroantimonate (223.74 Da) penetrates the intercellular spaces in excised gills of the fresh-water mussel *Elliptio complanatus*, presumably through septate junctions in the ctenidial epithelium (Satir and Gilula, 1970; see also Machin, 1977; Stommel, 1984a). And Uglem *et al.* (1985) demonstrated that molecules up to the size of inulin (5250 Da) would cross the pedal epithelium of the slug *Lehmannia valentiana* by a paracellular route with a molecular weight cutoff of 10^4 Da. Similarly, inulin seems to cross the ctenidia of the oyster *Crassostrea gigas* by a paracellular route (Hevert, 1984). Finally, DA, 5HT, AMSFYFPRMamide, and YFAFPRQamide all act upon the musculature of the labial palps of *Mercenaria* within several minutes (L. F. Gainey, unpublished observations). In conclusion, if we assume that the ctenidial epithelium of *Mercenaria* is similar to that of other molluscs, then DA, 5HT, and the SCPs could have entered the gills readily *via* a paracellular route, and the time for YFAFPRQamide to exert its effect on the lateral cilia would have been limited only modestly by the peptide's lower rate of diffusion.

The robust cardioexcitation, as well as other effects of the SCPs on various pulmonate and opisthobranch tissues, is accompanied by an increase in levels of cAMP (references in Reich *et al.*, 1997a, b). Furthermore, experiments on the accessory radula closer muscle of *Aplysia californica* (Probst *et al.*, 1994) and on isolated myocardial cells of *Helix aspersa* (Reich *et al.*, 1997a, b) showed that the peptide is exerting its effect by activating a cAMP-dependent protein kinase in the tissue. However, the isolated heart of *Mercenaria* responds only weakly and unreliably to the SCPs (Candelario-Martinez *et al.*, 1993); moreover, the strong mechanical responses of bivalve hearts to 5HT and FMRFamide may not, after all, be mediated by cAMP (reviewed by Bayakly and Deaton, 1992). Thus, the YFAFPRQamide antagonism of DA ciliary inhibition could be due to another mechanism, or the peptide could even be stimulating serotonergic neurons, releasing 5HT. Most evidence suggests that substance P also has such an indirect effect on mucociliary activity (*e.g.*, Lindberg and Mercke, 1986; Khan *et al.*, 1986; Wong *et al.*, 1991; Schlosser *et al.*, 1995).

Our experiments do not allow us to distinguish among the possibile mechanisms. But work with the gills of *Mytilus*

edulis showed that lateral cilia arrested by calcium ion—the likely mechanism of DA inhibition (Stommel 1984b; Stommel and Stephens, 1985a)—are stimulated to beat within seconds by 5HT and cAMP (Murakami and Takahashi, 1975; Stommel and Stephens, 1985b; for reviews of ciliary control mechanisms see Murakami, 1989, and Aiello, 1990). These data suggest that the much delayed onset of YFAFPRQamide action after the DA concentration has been raised from 5×10^{-7} *M* to 10^{-6} *M*, is due neither to 5HT release nor to augmented levels of cAMP.

The pharmacology of the frontal cilia is much more poorly known than that of the lateral cilia, and some of the data are contradictory. For example, reports agree that particle transport by the frontal cilia in *Mytilus* is stimulated by 5HT (Gosselin and O'Hara, 1961; Jorgensen, 1975; Malanga, 1975b). Yet 5HT also inhibits the "crawling" of isolated pieces of *Mytilus* gill that is effected by the frontal cilia (Malanga, 1975a). And species also matters, because, in contrast to the situation in *Mytilus*, particle transport by the frontal cilia of *Lampsilis* sp. is inhibited by 5HT (Malanga, 1975b). In this study, we found that 5HT inhibited the rate of particle transport in frontal cilia of *Mercenaria* in a dose-dependent manner. But we were unable to stop the transport completely, even with 10^{-3} *M* 5HT.

The physiological mechanisms underlying the control of the frontal cilia are also largely unexplained. Walter and Satir (1978) found that the frontal cilia of *Elliptio complanatus* are several orders of magnitude less sensitive to an influx of Ca^{++} than are the lateral cilia. Aiello (1979) reported that frontal ciliary activity in *Mytilus* increases in response to mechanical stimulation; similar results have been reported for the abfrontal cilia (Stommel and Stephens, 1988), where mechanical stimulation is accompanied by an influx of calcium. Thus, the mechanism of inhibition of frontal cilia by 5HT and the modulation of this response by the SCPs are probably different from those in lateral cilia. Frontal cilia remain difficult to work with, but the data reported here demonstrate that, since each ciliary tract has its distinct function (reviewed by Aiello, 1990), its physiology and regulatory mechanisms should also be distinct, a well-established principle with respect to muscles and other organs and tissues.

The lateral cilia often "vibrate" in an apparently uncoordinated manner, and then suddenly switch into well-defined metachronal waves [this report, and Catapane *et al.*, 1978 (*Mytilus edulis*)]; the waves are formed by the viscous coupling of cilia (reviewed in Satir and Sleigh, 1990). Now an examination of the data reveals that the lateral cilia in *Mercenaria* do not beat in a continuous range of frequencies; they especially do not beat in metachronal waves below 7 beats/s. The dose-response graph of DA (Fig. 1B) also reveals this behavior; *i.e.*, the cilia slow down, but only modestly, and then abruptly stop. A similar discontinuity is readily apparent in the DA (inhibitory) and 5HT (excitatory) dose-response curves for lateral cilia of *Mytilus* (Catapane, 1983). Assuming that 5HT and DA activate the initiation and cessation of ciliary beating, we suppose that YFAFPRQamide has the effect of smoothing out the transition between cessation and metachronal beating. This idea is supported by the following data from *Mytilus:* Stimulation of the branchial nerve with a single depolarizing pulse led to a single action potential on the lateral cells and complete cessation of ciliary beating, which lasted for as long as a second (Saimi *et al.*, 1983a). But stimulation of the branchial nerve at 25 to 50 Hz led to a decrease in, not an arrest of, the ciliary beat frequency; moreover, the cilia took 30 min to recover (Paparo and Aiello, 1970). Finally, there is strong evidence that the SCPs and acetylcholine are co-transmitters in *Aplysia* (Cropper *et al.*, 1987, 1990). Taken together, these data suggest that, in *Mercenaria* gills, DA and YFAFPRQamide are co-transmitters.

Candelario-Martinez *et al.* (1993) found that YFAFPRQamide and AMSFYFPRMamide are most concentrated in the labial palps and visceral ganglia, with substantial concentrations in the gills and gut. Moreover, immunoreactive, varicose fibers were found in all ganglia and peripheral tissues involved in feeding. Finally, YFAFPRQamide, and to a lesser extent AMSFYFPRMamide, caused relaxation of the gut. Based upon these findings, these authors proposed that one role of these SCP-related peptides in *M. mercenaria* is to regulate feeding and gut motility, as in a variety of gastropods (reviewed in Lloyd, 1989; Prior and Welsford, 1989; Weiss *et al.*, 1992). Our results are consistent with this hypothesis.

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